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CONTINUATION

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

JUL 30 1973

Application for Research Grant
(Use extra pages as needed)

Date July 24, 1973

1. Principal Investigator (give title and degrees):

Theodore Alan Slotkin, Ph.D., Assistant Professor of Pharmacology

2. Institution & address:

Dept. of Physiology and Pharmacology
Duke University
Durham, North Carolina 27710

3. Department(s) where research will be done or collaboration provided:

Dept. of Physiology and Pharmacology

4. Short title of study:

Maturation of the Adrenal Medulla: Catecholamine stores in normal and hypertensive rats.

5. Proposed starting date: January 1, 1974

6. Estimated time to complete: 1 year

7. Brief description of specific research aims: During the first weeks after birth, there are major changes in the catecholamine stores of the sympathetic neuron and its endocrine counterpart, the adrenal medulla. During the same period (5 weeks) spontaneously hypertensive Wistar rats (SHR) first show significantly elevated blood pressures. It has been reported that by the end of this period there is a change in catecholamine turnover in the SHR. It is proposed: (1) to study the maturation of adrenal catecholamine stores in SHR and normal rats, and (2) to elucidate the mechanisms by which changes in catecholamine turnover have occured. Techniques developed by this investigator will be used to measure the number of storage vesicles, their amine uptake and storage capabilities, and the degree to which stores can be mobilized during stress or depleted by drugs. These will include measurements of the amounts of vesicular components and the ability of the vesicles to incorporate isotopically labeled catechol- and non-catecholamines. Specifically, this study will attempt to determine the sequence and rate-limiting step(s) in the development of adrenal amine stores and to evaluate differences between normals and SHR in the development of the ability to maintain or secrete the amines. By identifying specific defects or changes in amine storage, these data could provide insight into the etiology of hypertension and into the interaction between hypertension and sympathetic nervous system function.

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8. Brief statement of working hypothesis: The development of hypertension is spontaneously hypertensive rats, in rats with surgically or pharmacologically induced hypertension, and in human essential hypertension, is associated with alterations in catecholamine storage. In spontaneously hypertensive rats, hypertension develops at a time when the catecholamine stores are undergoing marked development changes. It is proposed to study the process by which the stores increase during development in normotensive and hypertensive rats in order to define specific defects in sympatho-adrenal function.

9. Details of experimental design and procedures (append extra pages as necessary)

1. Previous work by applicant: The adrenal medulla is often utilized as a model of the sympathetic neuron; both tissues arise embryonically from the neural crest and both have the ability to synthesize, store and secrete catecholamines. Each contains storage vesicles which can accumulate amines by a mechanism which is stimulated by ATP - Mg^{2+} and blocked by reserpine. The vesicles contain dopamine beta-hydroxylase (DBO), chromogranins and adenine nucleotides as well as catecholamines; it is accepted generally that the catecholamines and adenine nucleotides (primarily ATP) form a storage complex in a molar ratio of 4 to 1.

For the past three years, the research of this investigator has been concerned with the development of sensitive and appropriate methods for the evaluation of the properties of the catecholamine storage vesicles of the adrenal medulla (1, 2, 3, 4). The adrenal medulla was chosen because it provides a more useful model than sympathetic nerves with which to study amine storage. Purified adrenal vesicles can be obtained in high yield by a relatively rapid discontinuous density gradient technique, while much lower yields of comparably purified sympathetic nerve vesicles are obtained after more lengthy procedures. Furthermore, the high concentration of storage vesicles in the adrenal permits the evaluation of properties which would be far more difficult to determine in nerve vesicles. Because of the development of these methods, the following parameters can be measured:

a. Uptake and storage capabilities of the vesicles. The simultaneous measurement of the accumulation of radioactively labelled amines by the vesicles along with the efflux of endogenous and labeled amines permits evaluation of these two parameters. The rate of efflux is determined by the stability of storage, while the accumulation is a measure of storage stability and affinity for uptake. Additionally, the relative importance of ATP - Mg^{2+} stimulated uptake can be evaluated by measuring the accumulation of metaraminol, an amine which is incorporated by a primarily ATP - Mg^{2+} -independent mechanism (1, 2).

b. Concentrations of intravesicular components.
Vesicles are purified by discontinuous sucrose density gradient centrifugation. The subcellular distributions of catecholamines, ATP and DBO can thus be readily determined, along with the fragility of the vesicles (see METHODS section). The buoyant density of the vesicles

can also be studied by continuous density gradient centrifugation, which provides a sensitive measure for evaluating small differences in the densities of different populations of vesicles (2, 4).

c. Secretion and recovery of amines and vesicles.
This can be evaluated using neurogenic secretion evoked by insulin-induced hypoglycemia or non-neurogenic amine loss produced by reserpine (3, 4).

Studies by this investigator utilizing these techniques have been published (1, 2, 3, 4). Experiments of this type were used to demonstrate the sequence of events during secretion and repletion of amine stores in normal adult rats, demonstrating that secretion of the vesicle contents is all-or-none, and that resynthesis of catecholamines is probably the rate-limiting step in repletion.

In addition, during the first half-year of this project, the techniques have been used to determine the maturational process in normal rats to serve as a base-line with which to compare SHR (see appended progress report) and to observe some of the alterations in the adrenal medulla of adult SHR. These studies suggest that altered neural input occurs in the SHR and that this may in turn slow maturation of the gland. Furthermore, determinations of the kinetic uptake constants suggest the possibility that the SHR may be somewhat more resistant to some antihypertensive agents (reserpine).

The first phase of the study - maturation in normal rats - has been completed, and preprints are appended describing the results in detail.

2. Previous work by other investigators: During prenatal and postnatal development, there is a marked increase in catecholamine levels in adrenergic neurons and in the adrenal medulla (5, 6, 7, 8), as well as changes in catecholamine synthesizing enzymes (5, 9). Although the necessary enzymes are present early in gestation (5), catecholamines do not appear until late in gestation, at a time when storage vesicles first become detectable (10, 11), suggesting that the storage vesicles play a determining role in the increase in adrenal amines. Consequently, the largest changes in catecholamine content occur in the postnatal period (0-6 weeks after birth). Spontaneously hypertensive Wistar rats (SHR) first show significantly elevated blood pressures towards the end of this period (5 weeks), along with disturbances in sympathetic catecholamine synthesis, storage and release (12). Similarly, in studies utilizing uninephrectomized rats treated with desoxycorticosterone acetate and NaCl, it has been shown that the resultant hypertension is accompanied by a defect in catecholamine storage such that cardiac storage vesicles become "leaky" (13). Westfall (14) has likewise demonstrated changes in catecholamine turnover in rats with elevated systolic blood pressures produced by chronic nicotine administration. Impairment of catecholamine storage has been implicated in essential hypertension in humans, as evidenced by increases in excretion of catecholamines and their metabolites in individuals with that disease (15).

In each case, hypertension was accompanied by a disturbance in sympathetic function probably involving impaired storage, Therefore, it is important to examine systematically the properties of the storage vesicles in at least one of the model systems. The SHR is probably the most reliable of all the models of hypertension to use for a study of this type : hypertension develops

rapidly and requires no pharmacological or surgical intervention, the animals are available as a genetically pure strain, and inbred normotensive Wistar rats provide a valid control (12). It is of additional interest that, although the mechanism of hypertension may be different from SHR, essential hypertension in humans is probably genetic in origin (16).

Utilizing the techniques developed by this investigator, this study will attempt to elucidate possible differences in the catecholamine stores of SHR and normal rats by examining the uptake, storage and release of amines from adrenal medullary vesicles during the period from birth until the development of hypertension.

3. Methods: Litters of SHR and normotensive Wistar rats will be sacrificed at intervals of several days over the period from birth until the development of hypertension in the SHR group (about 6 weeks) (12). The adrenal glands will be removed and analyzed as follows:

a. Determination of the number and contents of storage vesicles. The adrenal glands will be homogenized in isotonic sucrose, centrifuged at 800 x g for ten minutes, and the supernatant will be layered on 1.6 M sucrose and centrifuged for 2 hours at 140,000 x g. The latter centrifugation separates storage vesicles from most mitochondrial and lysosomal contaminants (17) as well as from broken vesicle membranes (3). All fractions will be assayed for catecholamines (CA) (trihydroxyindole method, 18), for ATP (firefly method, 19) and for dopamine beta-hydroxylase (DBO) (periodate oxidation method, 20). DBO is an enzyme associated with both the soluble and membrane-bound fractions of the storage vesicles (21), and the determination of DBO activity therefore provides an estimate of the number of storage vesicles present. The low levels of DBO present at the early stages of development may require the pooling of glands from several animals to obtain sufficient enzyme activity. The ratio of vesicular catecholamines to DBO provides a measure of the sequence of amines and vesicles: if CA/DBO remains constant during development, this would suggest that the rate-limiting step in development is vesicle synthesis. If CA/DBO increases, then vesicle synthesis is not rate-limiting. Thus, alterations in DBO levels in developing SHR may indicate changes in the number of storage vesicles present, while alterations in CA/DBO may indicate a change in the limiting step in age-dependent CA increases.

ATP is an integral part of the catecholamine storage complex. If CA/ATP is less than the adult ratio of 4 during the period of development, this would imply that nucleotide accumulation is not rate-limiting in establishment of amine stores. If CA/ATP is constant throughout development, then nucleotide accumulation may be rate-limiting. In developing hypertensive rats, alterations in ATP levels may indicate impaired storage capabilities.

The fraction of vesicles ruptured during homogenization is fairly constant from preparation to preparation (3, 4). Therefore, the ratio of DBO in the broken vesicle membrane fraction to the DBO in the intact vesicle fraction may provide a measure of "fragility" of the storage vesicles in both normotensive and hypertensive rats.

Alterations in any of the above factors--number of vesicles, ATP levels, vesicle fragility--could alter catecholamine storage.

b. Determination of the uptake and storage properties of the storage vesicles: Litters of rats will be sacrificed as described above, and adrenal homogenates will be centrifuged at 800 x g. Supernatants will then be used for determination of vesicular catecholamine fluxes. To determine the uptake capabilities of the vesicles, the suspensions will be incubated at 30° with either ^{14}C-epinephrine or ^{3}H-metaraminol, in the presence of ATP - Mg^{2+} as described previously (3, 4). The former amine is incorporated primarily by the reserpine-sensitive uptake mechanism, while the latter is incorporated primarily by the reserpine-insensitive mechanism (1, 2, 22, 23). The effluxes of endogenous and newly-incorporated amines from the storage vesicles will also be determined (1, 2, 3). Because "uptake" is a complex term (influx minus efflux), and since efflux is a measure of the stability of storage, only by the evaluation of efflux can an observed decrease in uptake be interpreted as a decrease in influx or a decrease in stability of storage (increase in efflux). These data should indicate whether there is a specific defect in uptake or storage of amines in hypertensive rats.

c. Buoyant density of storage vesicles: Catecholamines and nucleotides represent a significant fraction of the dry weight of the storage vesicles (24). Therefore, it would be expected that, if vesicles from hypertensive rats have altered CA or ATP levels, they might equilibrate at lower-than-normal densities on continuous sucrose gradients. The separation of lighter vesicles with lower CA contents in normal adult rabbits and rats after massive vesicle depletion has been described previously (4, 25); studies of this type will be carried out with vesicles from developing normotensive and hypertensive rats to see whether there are differences in buoyant density.

d. Depletion and repletion of adrenal amine stores: The ability of the adrenal glands of developing SHR and normal rats to respond to neural stimulation and to recover from massive stimulation will be tested by the administration of insulin (5 IU/kg); in normal adult rats, this results in depletion of adrenal CA to 20% of control levels within 4 hours (3), followed by a return to normal levels in 4 days (4). Should the ability to secrete amines be altered in the SHR, similar studies will be conducted in vitro using potassium as a secretogogue. If there is an in vitro response but only poor in vivo secretion after insulin, this could imply that the altered response to neural stimulation results from a presynaptic defect. If the gland responds poorly to both treatments, it would imply that any alteration in catecholamine secretion is due to a change in the ability of the adrenal to respond to neural input, either through interference with amine synthesis, storage, or secretion.

The rate of recovery of amines and vesicles after neural stimulation (insulin administration) or after non-neural depletion by reserpine (5 mg/kg) would give further information regarding whether the rate of CA and vesicle turnover is altered in hypertensive rats. For example, 50% of the vesicles lost during massive secretion are replaced within 24 hours in normotensive adult rats (4). It would therefore be worthwhile to study the rates of recovery in developing SHR and normotensive rats to determine if there is any alteration in the capacity to resynthesize vesicles which have been secreted.

Because of the likelihood of altered neural input in SHR, studies utilizing chlorisondamine (a ganglionic blocking agent) will be carried out: SHR and normals will be given twice daily injections (5 mg/kg s.c.) for one week and

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parameters which previously demonstrated differences will be measured. Since neural input will have been blocked, these differences will disappear if they were central (i.e. neural) in origin but not if the differences were inherent in the glands themselves.

Since preliminary studies suggest that altered sensitivity to reserpine and other uptake blockers (notably harmine) may occur in SHR, in vitro uptake studies will be conducted to determine sensitivity to these agents.

4. Significance: The sympathetic nervous system and its endocrine counterpart, the adrenal medulla, exert important regulatory functions on the entire cardiovascular system. During the first six weeks after birth, the adrenergic neurons and adrenal medullae of the rat undergo marked changes in catecholamine synthesis, uptake, storage and release. At the same time, hypertension begins to develop in spontaneously hypertensive Wistar rats which is associated with defects in the physiological disposition of sympathetic amines. The proposed study is designed to identify specific changes in the ability of the vesicles of the adrenal medulla to take up and store amines or to release them upon appropriate stimulation. The development studies could determine at what time after birth these changes occur. Only by direct measurement of the amounts of vesicular components and of vesicular properties can alterations of this type be identified. The developmental studies could also elucidate the nature of the process by which catecholamine stores increase during development and will indicate whether the rate-limiting step is the synthesis of vesicles, the synthesis of catecholamines, or the development of the ability of the vesicles to store the amines.

To summarize, the significance of the proposed study is that it may provide answers to the following basic problems:

a. Interaction of hypertension and the sympatho-adrenal system:
   1. Is there an altered catecholamine turnover rate in hypertensive animals?
   2. Is there an alteration in the ability to secrete amines upon stimulation?
   3. What defect(s) is (are) responsible for alterations in (1) and (2), on the vesicular and subvesicular levels?
   4. Do these alterations affect the sensitivity to antihypertensive agents?

b. Etiology of hypertension:
   1. Do alterations in catecholamine stores precede the development of hypertension?
   2. Can these alteration explain the hypertension?

c. Development of adrenal catecholamine stores:
   1. What processes occur during the postnatal period to cause the increase in adrenal catecholamines?
   2. Are these processes altered in hypertensive rats?

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parameters which previously demonstrated differences [illegible] be measured. Since

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20. O.H. Viveros, L. Arqueros and N. Kirshner, Mol. Pharmacol. 5: 342 (1969).
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10. Space and facilities available (when elsewhere than item 2 indicates, state location): Laboratory consists of 850 sq. ft. fitted with standard laboratory-type benches. Major items of equipment include Sorval RC-ZB centrifuge, Beckman L5-50 ultracentrifuge with rotors, Farrand ratio fluorometer, catecholamine autoanalyzer, Wang 600-6-T-P programmable calculator, incubation bath, pH meter, balances and general items of glassware and hardware. Research facilities and liquid scintillation spectrometer.

11. Additional facilities required: None

12. Biographical sketches of investigator(s) and other professional personnel (append):

13. Publications: (five most recent and pertinent of investigator(s), append list, and provide reprints if available).

Theodore A. Slotkin - Privileged Communication

CURRICULUM VITAE

NAME:

Theodore Alan Slotkin

BORN:

REDACTED

MARRIED:

REDACTED

CHILDREN:

REDACTED

SOCIAL SECURITY NUMBER:

REDACTED

EDUCATION AND DEGREES:

B.S. - Brooklyn College, CUNY, 1967

Ph.D. - Department of Pharmacology and Toxicology - Univ. of Rochester, 1970

POSITIONS HELD:

June 1971 - present	Assistant Professor, Dept. of Physiology and Pharmacology, Duke Univ. Medical Center
June 1970 - May 1971	Research Fellow, Dept. of Biochemistry, Duke Univ. Medical Center
February 1970 - May 1970	Postdoctoral Fellow, Dept. of Pharmacology and Toxicology, Univ. of Rochester

SOCIETIES:

REDACTED

RESEARCH ACTIVITIES:

Neurochemistry
Neuropharmacology

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BIBLIOGRAPHY

1. T.A. Slotkin and V. DiStefano, Urinary metabolites of harmine in the rat and their inhibition of monoamine oxidase. Biochem. Pharmacol. 19: 125-131, 1970.
2. T.A. Slotkin, V. DiStefano and W.Y.W. Au, Blood levels and urinary excretion of harmine and its metabolites in man and rats. J. Pharmacol. Exp. Ther. 173: 26-30, 1970.
3. T.A. Slotkin and V. DiStefano, A model of harmine metabolism in the rat. J. Pharmacol. Exp. Ther. 174: 456-462, 1970.
4. T.A. Slotkin and V. DiStefano, Cardiovascular and respiratory effects of harmine. Proc. Soc. Exp. Biol. Med. 133: 662-664, 1970.
5. T.A. Slotkin, R.M. Ferris and N. Kirshner, Compartmental analysis of amine storage in bovine adrenal medullary granules. Mol. Pharmacol. 7: 308-316, 1971.
6. T.A. Slotkin and N. Kirshner, Uptake, storage and distribution of amines in bovine adrenal medullary vesicles. Mol. Pharmacol. 7: 581-592, 1971.
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9. T.A. Slotkin and K. Edwards, The effects of reserpine on the content and properties of rat adrenal medullary storage vesicles. Biochem. Pharmacol. 22: 549-560, 1973.
10. N. Kirshner and T.A. Slotkin, Stimulating experiences with the adrenal medulla. Proc. 8th Midwest Conf. on Endocrinol. and Metabolism in press.
11. T.A. Slotkin, Maturation of the adrenal medulla. I. Uptake and storage of amines in isolated storage vesicles of the rat. Biochem. Pharmacol. in press.
12. T.A. Slotkin, Maturation of the adrenal medulla. II. Content and properties of catecholamine storage vesicles of the rat. Biochem. Pharmacol. in press.
13. T.A. Slotkin and N. Kirshner, Binding of amines to purified bovine adrenal medullary storage vesicle membranes. Biochem. Pharmacol. in press.
14. T.A. Slotkin, Reserpine, in "Neuropoisons: Their Pathophysiological Actions" vol. 2 (L.L. Simpson and D.R. Curtis, eds.) in press.
15. N. Kirshner and T.A. Slotkin, Secretion and recovery of catecholamines of the Adrenal Medulla. Biochem. Pharmacol. in press.
16. T.A. Slotkin, Hypothetical Model of Catecholamine Uptake into Adrenal Medullary Storage Vesicles. Life Sci. in press.

Theodore A. Slotkin - Privileged Communication

Abstracts:

1. T.A. Slotkin and V. DiStefano, Urinary metabolites of harmine in the rat and their inhibition of monoamine oxidase. Fed. Proc. 28: 797, 1969.
2. T.A. Slotkin, V. DiStefano and W.Y.W. Au, Metabolism of harmine in rats and humans. Pharmacologist 11: 273, 1969.
3. T.A. Slotkin and V. DiStefano, A model of harmine metabolism in the rat. Fed. Proc. 29: 678, 1970.
4. T.A. Slotkin, Efflux of ^{14}C-epinephrine from bovine adrenal medullary granules. Fed. Proc. 30: 445, 1971.
5. T.A. Slotkin and N. Kirshner, Structure-activity relationships for uptake and storage of amines by isolated bovine adrenal medullary vesicles. Pharmacologist 13: 228, 1971.
6. T.A. Slotkin and N. Kirshner, Depletion of rat adrenal medullary constituents following insulin. Fed. Proc. 31: 521, 1972.
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8. T.A. Slotkin, Uptake and storage of amines in isolated adrenal medullary vesicles of developing rats. Fed. Proc. 32: 783Abs, 1973.
9. T. A. Slotkin, Maturation of Adrenal Catecholamine Storage Vesicles of the Rat. Pharmacologist in press.

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Theodore A. Slotkin - Privileged Communication

CURRICULUM VITAE

Hannah O. Green

Date of Birth: October 14, 1935

Place of Birth:

REDACTED

Marital Status:

Education:

1953-1957	Carnegie Mellon University	B.S.	Chemistry
1958-1964	Cornell University	M.S.	Biochemistry
		Ph.D.	Biochemistry

Professional Experience:

6/57 - 6/58	Chemist, Jones and Laughlin Steel Corporation Research Laboratory, Pittsburgh, Pa.
9/64 - 12/66	Research Associate, Department of Biochemistry and Biophysics, University of Hawaii, Honolulu, Hawaii.
2/69 - 12/69	Research Associate, Department of Physiology and Pharmacology, Duke University Medical Center, Durham, N.C.
8/70 - ~~present~~ 9/71	Research Associate, Department of Biochemistry, Duke University Medical Center, Durham, N.C.

Publications:

Oppenheimer*, H. L., J. Mercouroff, and G. P. Hess, Biochim. Biophys. Acta, 71, 78 (1963). "Characterization of the Difference Spectrum of Diisopropylphosphoryl-α-chymotrypsin versus α-Chymotrypsin. IV. The Environment of Tryptophyl Residues."

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* Maiden name

Publications (continued)

Labouesse, B., K. Carlsson, H. L. Oppenheimer, and G. P. Hess in "Structure and Activity of Enzymes" (Goodwin, T. W., J. I. Harris, and B. S. Hartley, editors), Academic Press, New York, 1964, p. 71. "Characterization of a Residue Controlling the Activity and Conformation of Chymotrypsin."

Oppenheimer, H. L., B. Labouesse, K. Carlsson, and G. P. Hess, Federation Proceedings, 23, 315 (1964). "Role of N-Terminal Isoleucyl Group in Conformation and Activity of Chymotrypsin."

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Green, H. O., J. Moritz, and L. Lack, Biochim. Biophys. Acta, 231, 550 (1971). "Binding of Sodium Taurocholate by Bovine Serum Albumin."

Green, H. O. and J. A. Reynolds, Federation Proceedings, 30, 1065 (1971). "Protein Components of Porcine Brain Myelin."

References:

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Dr. Leon Lack
Department of Physiology and Pharmacology
Duke University Medical Center
Durham, North Carolina 27706

Dr. Jacqueline A. Reynolds
Department of Biochemistry
Duke University Medical Center
Durham, North Carolina 27706

Publications from this Project

1. Maturation of the adrenal medulla. I. Uptake and storage of amines in isolated storage vesicles of the rat. T.A. Slotkin, Biochem. Pharmacol. in press.
2. Maturation of the adrenal medulla. II. Content and properties of catecholamine storage vesicles of the rat. T.A. Slotkin, Biochem. Pharmacol. in press.
3. Binding of amines to purified bovine adrenal medullary storage vesicle membranes. T.A. Slotkin and N. Kirshner. Biochem. Pharmacol. in press.
4. Secretion and recovery of catecholamines from the adrenal medulla. N. Kirshner and T.A. Slotkin, Biochem. Pharmacol. in press.
5. Hypothetical model of catecholamine uptake into adrenal medullary vesicles. T.A. Slotkin, Life Sci. in press.
6. Reserpine-like effects of harmine on adrenal medullary storage vesicles. H.O. Green and T.A. Slotkin, submitted for publication.

Abstracts:

1. T. A. Slotkin, Uptake and storage of amines in isolated adrenal medullary vesicles of developing rats. Fed. Proc. 32: 783 Abs. (1973).
2. T. A. Slotkin, Maturation of Adrenal Catecholamine Storage Vesicles of the Rat. Pharmacologist, in press.

14. First year budget:

A. Salaries (give names or state "to be recruited")

Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
Theodore A. Slotkin	60	
Hannah Green	100	REDACTED
Fringe Benefits @ 12.10%		
Technical		
	Sub-Total for A	REDACTED

B. Consumable supplies (by major categories)

Rats - 1000 @ $2.00		2,000
Animal housing and shipping		500
Isotopes		1,500
Chemicals and hardware		1,000
	Sub-Total for B	$ 5,000.00

C. Other expenses (itemize)

Equipment maintainence and service		500
Travel to FASEB and ASPET meetings		500
	Sub-Total for C	$ 1,000.00
	Running Total of A + B + C	$ REDACTED

D. Permanent equipment (itemize)

	Sub-Total for D	
E. Indirect costs (15% of A+B+C)	E	$ 1,741.
	Total request	REDACTED

15. Estimated future requirements.

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2						
Year 3						

1003541949

16. Other sources of financial support-
List financial support from all sources, including own institution, for this and related research projects

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Adrenal catecholamine fluxes in SHR	N.C. Heart Ass. 1972-73 A-10	2,500	1-15-73 -- 1-14-74
Catecholamine stores in SHR	Duke University Inman Fund	3,750	7-1-73 -- 6-30-74

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Amine stores of developing normal and hypertensive rats.	American Heart Ass.	50,000	requested for 9-1-73 -- 8-31-76

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made"

Principal investigator

Typed Name Theodore Alan Slotkin, Ph.D.

Signature [signature] Date 7/23/73

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Responsible officer of institution

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